A Broader Perspective of Sexual Differentiation

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"But if I wish to define myself, I must first of all say: "I am a woman;" on this truth must be based all further discussion. A man never begins by presenting himself as an individual of a certain sex; it goes without saying that he is a man."

The Second Sex
Simone De Beauvoir, 1953

Human sexual differentiation is customarily depicted as a series of embryonic events that lead to male and female gonadal development and differential hormone expression that have behavioral as well as biological outcomes. The salient components of these events are the differential expression of two hormones—testosterone and Müllerian inhibiting substance—and the *SRY* gene, regulating, in turn, the transcription of other genes and culminating in male differentiation. Sex determination, then, is generally described as initially proceeding down a path toward female development unless the bipotential, indifferent gonad is modified toward male development by genes on the Y chromosome.

In this issue of the *American Journal of Medical Genetics,* Lubinsky hypothesizes that disturbances of determination and morphogenesis account for sex biases of various congenital malformations [1997]. His analysis that there are underlying developmental relationships among the anomalies associated with each sex has considerable clinical import to clinical geneticists. The next question, of course, is what might lie behind the trend that female-associated malformations appear to be tissue and patterning anomalies and the trend behind male-associated malformations is toward structural problems? And the intuitive response is to seek possible answers or theories from what we already know about sexual differentiation and/or developmental differences between the sexes expressed during the embryonic and fetal periods.

While we were tempted to offer a speculative explanation for Lubinsky's observations, we found that his insightful analysis leads to another question first. . . . "Is the current understanding of sexual differentiation too narrow a concept to offer an explanation for Lubinsky's data and analysis?" Generally, the concepts of "maleness" and "femaleness" are reflected by such terms as "complementary" or "opposites," as in "opposite sex." And in one way or another, these terms certainly suffice when thinking about the most obvious functions of sexual differentiation, namely, mating and procreation. But, perhaps, this is too restricted a way to contemplate and understand sex differentiation and differences. Lubinsky's work stimulates us to consider sex differentiation in a broader perspective.

A growing number of biological events in mammalian development are recognized as being predominantly associated with one sex or the other: imprinting [Hall, 1990; Thornhill and Burgoyne, 1993]; expression of *SRY*/*sry* prior to gonadal development [Zwingman et al., 1993; Ao et al., 1994; Fiddler et al., 1995]; X-chromosome inactivation; differential rates of mutation [Penrose, 1955; Vogel, 1977]; differential rates of crossing over [Burt et al., 1991]; differential presence of H-Y antigen in preimplantation embryos [Epstein et al., 1981]; differential embryonic growth [Xu et al., 1992; Pergament et al., 1994]; differential development of malformations [Arena and Smith, 1978; Lubinsky, 1997; differential risk of transmitting a chromosome 14/21 translocation [Lister and Frota-Pessoa, 1980]; differential development of brain structures (non-hormonally related) [Reisert and Pilgrim, 1991]; genetic contributions to male homosexuality [Hu et al., 1995]; differential development of lung surfactant [Nielsen and Torday, 1985]; and, differential metabolic rates [Lentner, 1981; Ray et al., 1995].

These citations are meant to be exemplary and, more importantly, not inclusive of biological events understood to be the outcomes of gene actions leading to sex organ development or of established hormonal effects. So, rather than make Lubinsky's compilations and interpretation the object of further speculation, we suggest that they be placed alongside these other phenomena and contemplated as one of many data sets that beg for explanation in the context of sexual differentiation. The question we wish to address is whether the observations regarding differential sex effects must ultimately be reconciled by a model of sexual differentiation that is sufficiently robust to offer explanation for these diverse phenomena. Is it time to reconsider the characterization of "femaleness" and "maleness?"

We propose that sex-related and/or sex-biased traits,

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in addition to being the result of underlying sex determining pathways, are themselves the direct expressions of sexual differentiation. Sexual differentiation, then, may be considered a spectrum of events that begins in the preimplantation stage of embryonic development, if not at the time of conception [Epstein et al., 1980; Zwingman et al., 1993; Pergament et al., 1994; Ao et al., 1994; Fiddler et al., 1995], and continues systematically throughout development. From a phenotypic perspective, successful differentiation may rest on a threshold of gonadal development but also comprise a series of diverse biologic/genetic phenomena culminating in a continuum between biological maleness and femaleness; i.e., in addition to gonadal and anatomical development, the sexes are also characterized by the combination and integration of such phenomena as imprinted genes, rates of crossing over, variation in organ and tissue development (extremes of which result in malformations as described in Lubinsky's report), metabolic rates, and/or growth rates. Each of these phenomena exhibits variation toward or away from the aggregate sex bias, contributing to the spectrum of "maleness" and "femaleness."

The heterogametic nature of mammalian organisms has typically directed the discussion of sex determination toward elucidating the functions and mechanisms of Y-borne genes. However, Clarke and Mittwoch [1995] have suggested that sex differentiation, sex ratios and longevity may be better understood as related processes that start at conception and are primarily characterized by differential growth rates favoring the heterogametic embryo. Genes involved in testis development, then, may function as growth regulators in non-gonadal tissues in which they are active [Mittwoch, 1992]. It may be that sex-based or sex-biased differences are the result of a Y-borne gene system acting early, and perhaps constitutively, as a fulcrum in a branching cascade and linking gene and environmental interactions to the myriad of sex-biased or limited events. However, it is more likely that other genes are critical to these sex differentiated outcomes.

The emerging genotypic complexity of sex determination has already pointed to the involvement of genes that may or may not be related to the activity of SRY, the "testes determining factor." In humans, there are genes on the X chromosome and autosomes causing sex reversal (i.e., XY females, "Swyer syndrome") and SRY-negative, XX maleness. The presence of autosomal sex-determining genes in humans and mice has also been advanced [Eicher, 1988; Washburn et al., 1990; McElreavy et al., 1995]. According to Mittwoch [1992], male sex differentiation in XY individuals can be impaired by an euploid but "inappropriate" genetic background. The presence of autosomal genes affecting sex determination is illustrated by the skeletal malformation syndrome, campomelic dysplasia (CD), and the associated autosomal XY sex reversal caused by mutations in and around the SRY-related gene, *SOX9,* on chromosome 17 [Tommerup et al., 1993; Wagner et al., 1994]. Mutant *SOX9* proteins with an intact HMG (high mobility group) domain but lacking the TA (transcription activation) domain are stable. The *SOX9* protein binds to target DNA sequences in the chondrogenic as well as testogenic pathways; CD mutations can still bind but are apparently unable to effectively transactivate target genes. The result is CD and sex reversal [Sudbeck et al., 1996]. The relationship of these SOX9 mutations to the actions of SRY, however, remains unknown.

Other genes having possible roles in sex differentiation may be related to each other through the evolution of the Y chromosome, e.g., *ZFX* and *ZFY,* murine *Ube1* and *Ube1Y, SOX3* and *SRY* [Graves, 1995]. While these and other genes involve relationships between the X and Y chromosome in the pseudoautosomal region, there are several autosomal genes that appear to have Y chromosome counterparts, presumably reflecting structural evolution of the Y chromosome [Graves, 1995]. In an analogous manner, genes may have been selected because of their molecular integration into coordinated developmental sequences leading to male or female phenotypes; allelic forms of these same genes contribute not only to the clinical features characterizing disturbances in sex differentiation but to a broad, overlapping spectrum encompassing maleness and femaleness. Unraveling the involvement and the impact of these genes, particularly those of autosomal loci and/or those connected to sex-biased characteristics, should lead to models that notably expand current concepts of sexual differentiation. The key question is whether a set of critical genes primarily: 1) directs gonadal determination from which other sex differences are secondary outcomes; 2) initiates a broad set of events that are exemplified by those listed previously, of which gonadal development is one; and/or 3) functions systematically in concert with other genes to produce gonadal and non-gonadal sex differences.

Testing the hypothesis that sexual differentiation is a continuum of variation may be a matter of experimentation and of observation of mutations. The eventual elucidation of the mechanisms and molecular biology of each sex associated phenomenon will be the most important source of evidence. This may require that processes with sex differences be studied separately in each sex and comparatively between them. The hypothesis would be validated if critical sex-differentiating actions could also be dissociated from gonadal development and connected to such events as crossing over, growth rates, or tissue development. The extent to which the SRY gene, alone or through genetic intermediaries, is involved in sex-biased phenomena may be pivotal in discriminating between the first two possible functions described above of a gene system critically involved with sexual differentiation.

Defining the role and effects of putative non-SRY male determining genes will also add substantially to understanding the extent of the relationship of sexual differentiation and the sex-biased phenomena described by Lubinsky, particularly if their gene actions extend beyond testes development. Furthermore, insight into the biological events associated with female development, and their clinical consequences, must complement any test of the hypothesis that sexual differentiation is a continuum of variation. The DSS gene (dosage sensitive sex reversal) on the X chromosome may be an example of a gene that activates expression of other genes requisite for formation of female struc-

tures; it is also possible that this gene, when duplicated against the background of an intact SRY, may repress the SRY-induced male pathway [Bardoni et al, 1994]. However, the significance of DSS lays with the recognition that female developmental pathways should be explored further. An expanded concept of maleness and femaleness may emerge from following the path of male differentiation but it is also likely that viewing female development on its own terms will contribute to a more complete picture of sexual differentiation. Lubinsky [1997] suggested this as well by noting that differences in sex biases for congenital anomalies point to different developmental processes. Overall, sex differences existing in the expression of any genetic or biologic process should be exploited to expand the concept of maleness and femaleness. Individuals with different forms of sex reversal should be examined, for example, for expression of imprinted genes discordant with the transmitting parent. Such an observation would be consistent with the possibility that characteristics forming a spectrum of maleness/femaleness may be related, yet distinct, from gonadal differentiation.

Clearly, new insights into the relationships among genetic sex (*SRY* and the consequences of its expression), chromosomal sex (XX-XY), and phenotypic sex are needed and would have implications that range from basic biology to health care. Expanding the concept of sex differentiation also highlights the evolution of the X and Y chromosomes and their integration into the genome as part of normal molecular and developmental processes. The complex origin of the Y chromosome from the X chromosome [Graves, 1995], including assumption of male-specific functions, is compatible with the concept that sexual differentiation represents only part of the phenotypic variation characterizing maleness and femaleness. However, to be complete, any model of maleness/femaleness will also have to integrate the observations of Lubinsky and many others as part of the non-linear and interactive biological events characterizing sexual differentiation. Ultimately, the emergence of a more accurate picture of human nature is likely to require not only rethinking and redefining biological concepts of sexual differentiation and development but their synthesis with psychosocial concepts as well.

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